METHODS AND SYSTEMS FOR DETERMINING SYNAPSE FORMATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 62/743,153, filed Oct. 9, 2018, the contents of which are incorporated by reference in their entirety.

FIELD OF INVENTION

[0002] The presently disclosed subject matter relates to methods and systems for determining synapse formation, e.g., synapse formation associated with the activity of multispecific antibodies such as T cell-dependent bispecific antibodies.

BACKGROUND

[0003] Multi specific antibodies, such as bispecific antibodies, are important as research tools, diagnostic tools and as therapeutics. This is due, in large part, to the fact that such antibodies can be selected to bind with high specificity and affinity to two or more antigens or two or more epitopes present on an antigen. For example, in the case of cancer therapeutics, multispecific antibodies can be used to target a cancer cell, e.g., by binding an antigen present on the cancer cell, to an immune cell to trigger an immune response. In addition, multispecific antibodies can be used as ligands for heterodimeric receptors that are normally activated by their cognate ligand when it binds to and promotes interaction between the components of the receptor.

[0004] Targeting tumor-associated cell surface antigen with therapeutic monoclonal antibodies (mAbs) or antibody-drug conjugates (ADCs) has been shown to be very effective for the treatment of hematological and solid tumor malignancies. These molecules often rely on one or combinations of the following mechanisms of action (MOA) to kill tumor cells: antibody-dependent cell-mediated cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), receptor blockade, or internalization and intracellular release of conjugated cytotoxic drug. Despite the differences in MOA, maximizing/optimizing target engagement along with the consideration of pharmacokinetic properties of the therapeutic molecules has been an important driver for dose/regimen decision. Accordingly, there is a need for methods and systems for maximizing/optimizing mAbs and ADCs for treating cancer.

[0005] T cell-dependent bispecific molecules (e.g., bispecific T cell engager (BiTE) and T cell dependent bispecific antibody (TDB)), as a subset of multispecific antibodies, represent an emerging and promising class of therapeutic molecules for the treatment of cancer. Blinatumomab, a CD3xCD19 BiTE, has been proven effective for the treatment of a rare form of acute lymphoblastic leukemia and recently received an accelerated approval by the FDA (Bargou, R., E. Leo, et al. (2008) "Tumor regression in cancer patients by very low doses of a T cell-engaging antibody." Science 321: 974-977). Multiple novel T cell-dependent bispecifics are also in clinical development and have shown promising preliminary result. Accordingly, there is a need for methods and systems for developing and screening novel multispecific molecules for therapeutic use. Multiple novel T cell-dependent bispecifics are also in clinical development and have shown promising preliminary result. (Budde, L E, et al. (2018) "Mosunetuzumab, a Full-Length Bispecific CD20/CD3 Antibody, Displays Clinical Activity in Relapsed/Refractory B-Cell Non-Hodgkin Lymphoma (NHL): Interim Safety and Efficacy Results from a Phase 1 Study", Blood 132: 399).

SUMMARY OF THE INVENTION

[0006] The presently disclosed subject matter provides methods and systems for determining synapse formation, e.g., by screening multispecific antibodies (such as T cell-dependent bispecific (TDB) antibodies). In certain embodiments, the methods relate to screening a multispecific antibody, e.g., a T cell-dependent bispecific antibody, that is capable of inducing cellular synapse formation. In certain embodiments, the method comprises (a) contacting a multispecific antibody that binds to a first antigen and a second antigen with a first cell expressing the first antigen and a second cell expressing the second antigen, wherein a cellular synapse is formed between the first cell and the second cell upon binding of the multispecific antibody to the first and second antigens and (b) measuring activation of the first cell by the cellular synapse, and detectable activation of the first cell indicates that the multispecific antibody is capable of inducing cellular synapse formation.

[0007] The presently disclosed subject matter further provides methods of detecting cellular synapse formation. In certain embodiments, the method comprises (a) contacting a multispecific antibody that binds to a first antigen and a second antigen with a first cell expressing the first antigen and a second cell expressing the second antigen, wherein a cellular synapse is formed between the first cell and the second cell upon binding of the multispecific antibody to the first and second antigens; and (b) measuring activation of the first cell by the cellular synapse, and wherein detectable activation of the first cell indicates cellular synapse formation.

[0008] In certain embodiments, the multispecific antibody is a bispecific antibody. In certain embodiments, measuring activation of the first cell comprises measuring at a biomarker indicative of activation. In certain embodiments, the biomarker is a cell surface molecule. In certain embodiments, the biomarker is selected from the group consisting of CD62L, CD69, CD154, and combinations thereof. In certain embodiments, the biomarker is the expression of CD62L. In certain embodiments, the first cell is a T cell or a cell derived from a T cell. In certain embodiments, the first cell has a deficient cytolytic ability upon activation. In certain embodiments, the first cell is a Jurkat cell. In certain embodiments, the first antigen is CD3.

[0009] In certain embodiments, the second antigen is a tumor antigen. In certain embodiments, the tumor antigen is selected from the group consisting of HER2, LYPD1, LY6G6D, PMEL17, LY6E, EDAR, GFRA1, MRP4, RET, Steap1, TenB2, CD20, FcRH5, CD19, CD33, CD22, CD79A and CD79B. In certain embodiments, the second cell is a B cell. In certain embodiments, the tumor antigen is selected from the group consisting of CD20, FcRH5, CD19, CD33, CD22, CD79A and CD79B.

[0010] In certain embodiments, measuring activation of the first cell comprises detecting a reporter that is induced upon the activation of the first cell. In certain embodiments, the reporter is a fluorescent or luminescent molecule.